

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

1. (Original) A tangential flow filtration device for preparing a cell population enriched for leukocytes, comprising:

a remover unit having a cross-flow chamber, a filtrate chamber and a filter disposed therebetween, the filter in fluid communication with the cross-flow chamber and the filtrate chamber;

the cross-flow chamber having an inlet and an outlet, the inlet disposed to introduce a sample of blood constituents comprising leukocytes into the cross-flow chamber and parallel to the surface of the filter; and the outlet centrally disposed in a portion of the cross-flow chamber opposite the filter surface;

the filter having an average pore size ranging from about 1 to about 10 microns; such that flow of the sample across the filter enriches the sample of blood constituents for leukocytes.

2. (Original) The device according to claim 1, further comprising:

a means for providing a predetermined input rate of the sample to the inlet of the cross-flow chamber;

a means for controlling a filtration rate of filtrate through the filter and into the filtrate chamber; and

wherein the filtration rate controlling means limits the filtration rate to less than the unopposed filtration rate for the filter.

3. (Original) The device according to claim 1 or claim 2, wherein the filter pore size is about 3 microns to about 7 microns.

4. (Original) The device according to claim 1 or claim 2, wherein the filter pore size is about 3 microns to about 5.5 microns.

5. (Original) The device according to claim 1 or claim 2, further comprising: a source of blood constituents in fluid communication with the cross-flow chamber inlet.

6. (Original) The device according to claim 5, wherein the source of blood constituents is a leukopheresis device.

7. (Original) The device according to claim 1 or claim 2, further comprising: a recovery unit comprising an inlet and an outlet, the cross-flow chamber and the recovery unit interconnected in loop format, wherein the cross-flow chamber inlet is in fluid communication with the recovery unit outlet, and the cross-flow chamber outlet is in fluid communication with the recovery unit inlet.

8. (Original) The device according to claim 7, wherein the recovery unit further comprises a sample inlet and a wash inlet.

9. (Original) The device according to claim 8, further comprising a source of replacement liquid in fluid communication with the wash inlet.

10. (Original) The device according to claim 9, wherein the replacement liquid is an isotonic buffer or tissue culture media.

11. (Original) The device according to claim 1 or claim 2, wherein the cross-flow chamber is cylindrical and the outlet is located opposite the center of the filter and perpendicular to a surface of the filter.

12. (Original) The device according to claim 1 or claim 2, further comprising a

cell-processing apparatus in fluid communication with the remover unit.

13. (Original) The device according to claim 12, wherein the cell processing apparatus comprises beads.

14. (Original) The device according to claim 12, wherein the cell processing apparatus comprises a means for culturing the cell population enriched for leukocytes.

15. (Original) The device according to claim 14, wherein the means for culturing comprises:

a vessel having a first port and a second port;

a monocytic dendritic cell precursor adhering substrate, the substrate in fluid communication with the first port and the second port;

a screen for retaining the substrate within the vessel, the screen having a pore size sufficient to allow passage of monocytic dendritic cell precursors and dendritic cells therethrough;

a drain line in fluid communication with the first port; and

a collection line in fluid communication with the first port.

16. (Original) The device according to claim 15, further comprising a plurality of fluid sources in fluid communication with the first port or the second port.

17. (Original) The device according to claim 15, further comprising a sealable tissue culture vessel adapted to aseptically receive the monocytic dendritic cell precursors.

18. (Original) The device according to claim 17, wherein the sealable tissue culture vessel is a tissue culture bag, flask or bioreactor.

19. (Original) The device according to claim 15, wherein the fluid sources

comprise binding media, washing buffer and elution buffer.

20. (Original) The device according to claim 15, further comprising a pump in fluid communication with the plurality of fluid sources and the first port.

21. (Original) The device according to claim 15, further comprising: a temperature control means to maintain the substrate at a predetermined temperature.

22. (Original) The device according to claim 21, wherein the temperature controlling means is a heater.

23. (Original) A tangential flow device for enriching a sample of blood constituents for leukocytes, comprising:

a remover unit comprising a cross-flow chamber (3) and a filtrate chamber (4) separated by a filter (5), wherein the cross-flow chamber (3) has an inlet (6) and an outlet (7), the outlet centrally disposed in an upper portion of the chamber, and wherein the inlet is disposed above the filter and introduces fluid into the cross-flow chamber substantially parallel to the filter;

a means for providing a predetermined input rate (14) of the sample through the cross-flow chamber inlet; and

a means for reducing a filtration rate (15) through the filter; wherein the filter has a pore size of about 3 microns to about 7 microns; and whereby the sample is enriched for leukocytes in a retentate in the cross-flow chamber.

24. (Original) A tangential flow device for enriching a sample comprising blood constituents for leukocytes, comprising:

a remover unit (1) having a cross-flow chamber (3) and a filtrate chamber (4) separated by a filter (5), the cross-flow chamber having an inlet (6) and an outlet (7), the outlet

disposed above the inlet and centrally disposed in an upper portion of the chamber, and wherein the filter is disposed below and substantially parallel to the cross-flow chamber inlet;

means for providing a predetermined input rate (14) of the sample through the cross-flow chamber inlet;

means for providing a predetermined filtration rate (15) of the fluid through the filter, wherein the predetermined filtration rate is about one-fifth to about one one-hundredth of the predetermined input rate; and

means for providing a predetermined concentration of blood cells in the sample, wherein the predetermined concentration of blood cells is about  $10 \times 10^7$  to about  $10 \times 10^{10}$  cells per milliliter;

wherein the filter has pores having a pore size of about 3 microns to about 7 microns; and

whereby the sample is enriched for leukocytes in a retentate in the cross-flow chamber.

25. (Original) A method for separating leukocytes from a sample of blood constituents from a subject wherein the sample comprises leukocytes, the method comprising:

(1) introducing the sample into a remover unit through an inlet in the remover unit;

(2) subjecting the sample to cross-flow substantially parallel to a filter having a pore size of about 1 to about 10 microns;

(3) subjecting the fluid to filtration through the filter; and

(4) selectively removing non-leukocyte blood constituents from the sample to form a cell population enriched for leukocytes.

26. (Original) The method according to claim 25, further comprising:

preparing the sample from the subject by leukopheresis, density centrifugation, differential lysis, filtration, or preparation of a buffy coat, for introduction in the remover unit.

27. (Original) The method according to claim 25, wherein the non-leukocyte blood constituents include plasma and platelets.

28. (Original) The method according to claim 25, wherein the non-leukocyte blood constituents include erythrocytes.

29. (Original) The method according to claim 25, wherein the leukocytes comprise monocytes.

30. (Original) The method according to claim 25, further comprising repeating steps (1), (2), and (3) at least two times to form cell population enriched for leukocytes.

31. (Original) The method according to claim 25, wherein the enriched cell population comprises at least about 20% leukocytes.

32. (Original) The method according to claim 25, wherein the enriched cell population comprises at least about 60% leukocytes.

33. (Original) The method according to claim 25, further comprising inducing a vortex motion of the sample in the cross-flow chamber.

34. (Original) The method according to claim 25, further comprising washing the cell population enriched for leukocytes with a wash solution.

35. (Original) The method according to claim 25, further comprising preparing monocytic dendritic cell precursors from the cell population enriched for leukocytes.

36. (Original) The method according to claim 35, wherein the isolation of monocytic dendritic cell precursors comprises:

contacting a monocytic dendritic cell precursor adhering substrate with the cell population enriched for leukocytes;

allowing monocytic dendritic cell precursors in the cell population to reversibly adhere to the substrate to form complexes comprising monocytic dendritic cell precursors and substrate;

separating the complexes from the non-adhering leukocytes to obtain complexes comprising monocytic dendritic cell precursors; and

culturing the monocytic dendritic cell precursors to differentiate the precursors to form immature or mature dendritic cells.

37. (Original) The method according to claim 36, wherein the monocytic dendritic cell precursors are eluted from the substrate prior to culturing.

38. (Original) The method according to claim 36, wherein the monocytic dendritic cell precursors are cultured on the substrate.

39. (Original) The method according to claim 36, wherein the substrate comprises glass, polystyrene, plastic or glass-coated polystyrene microbeads.

40. (Original) A method for enriching a sample of blood constituents for leukocytes, comprising:

(1) introducing the sample into a tangential flow filtration (TFF) unit, the TFF unit comprising a cross-flow chamber, a filtrate chamber, and a filter in fluid communication with the cross-flow chamber and the filtrate chamber, the filter having a pore size of about 1 to about 10 microns;

(2) recirculating the sample through the TFF unit at a predetermined input rate and a predetermined filtration rate, the predetermined input rate at least five times the predetermined filtration rate; wherein the predetermined filtration rate is less than the unopposed filtration rate for the filter; and

(3) isolating a cell population enriched for leukocytes.

41. (Original) The method according to claim 40, wherein the enriched cell population is substantially free of non-leukocyte blood constituents.

42. (Original) The method according to claim 40, further comprising:  
collecting blood from a subject and preparing the sample from the blood by leukopheresis, density centrifugation, differential lysis, filtration, or preparation of a buffy coat.

43. (Original) The method according to claim 40, wherein the non-leukocyte blood constituents include plasma and platelets.

44. (Original) The method according to claim 41, wherein the non-leukocyte blood constituents include erythrocytes.

45. (Original) The method according to claim 40, wherein the leukocytes comprise monocytes.

46. (Original) The method according to claim 40, wherein the enriched cell population comprises at least about 20% leukocytes.

47. (Original) The method according to claim 40, wherein the enriched cell population comprises at least about 60% leukocytes.

48. (Original) The method according to claim 40, wherein the sample flows in a vortex motion in the cross-flow chamber.

49. (Original) The method according to claim 40, further comprising washing the enriched cell population with a wash solution.



50. (Original) The method according to claim 40, further comprising preparing dendritic cells from the enriched cell population.

51. (Original) The method according to claim 50, wherein the dendritic cells are prepared by:

contacting a monocytic dendritic cell precursor adhering substrate with the enriched cell population;

allowing monocytic dendritic cell precursors in the enriched cell population to reversibly adhere to the substrate to form complexes comprising monocytic dendritic cell precursors and substrate;

separating the complexes from the non-adhering leukocytes to obtain complexes comprising monocytic dendritic cell precursors; and

culturing the monocytic dendritic cell precursors to differentiate the precursors to form immature or mature dendritic cells.

52. (Original) The method according to claim 51, wherein the substrate comprises glass, polystyrene, plastic or glass-coated polystyrene microbeads.

53. (Original) The method according to claim 51, further comprising isolating the immature or mature dendritic cells.

54. (Original) The method according to claim 45, wherein the monocytes are cultured with cytokines that promote the differentiation of monocytes into dendritic cells.

55. (Original) The method according to claim 54, wherein the cytokines are GM-CSF, GM-CSF and IL-4.

56. (Original) The method according to claim 54, wherein the dendritic cells are

matured to mature dendritic cells.

57. (Original) The method according to claim 54, wherein the dendritic cells are cultured with an antigen under conditions conducive for processing the antigen to form antigen loaded dendritic cells.

58. (Original) The method according to claim 57, further comprising the step of administering the antigen loaded dendritic cells to an individual.

59. (Original) The method according to claim 57, wherein the antigen loaded dendritic cells are cultured with a maturation agent to mature the cells into mature antigen presenting dendritic cells.

60. (Original) The method according to claim 40, wherein the filter has a pore size of about 3 to about 5.5 microns.

61. (Original) The method according to claim 60, wherein the leukocytes comprise CD34<sup>+</sup> cells.

62. (Currently amended) The method according to claim 61, wherein the sample of blood constituents is from a donor ~~that~~ that has been treated with at least one stem cell mobilizing agent.

63. (Original) The method according to claim 62, wherein the stem cell mobilizing agent is G-CSF or cyclophosphamide.

64. (Original) The method according to claim 61, further comprising enriching the leukocytes for the CD34<sup>+</sup> cells.

65. (Original) The method according to claim 64, wherein the enrichment of leukocytes for the CD34<sup>+</sup> cells comprises using an anti-CD34 antibody conjugated to magnetic beads.

66. (Original) The method according to claim 61, further comprising expanding the CD34<sup>+</sup> cells *ex vivo*.

67. (Original) The method according to claim 60, further comprising preparing monocyte-derived pluripotent stem cells from the cell population enriched for leukocytes.

68. (Original) The method according to claim 60, further comprising inducing differentiation of a progenitor or stem cell.

69. (Original) The method according to claim 60, further comprising inducing transdifferentiation of a differentiated cell.